EXPRESS MAIL MAILING LABEL
EXPRESS MAILING NO.: EL 794535200 US
DATE OF DEPOSIT: February 25, 2002

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Peter DROGE, Nicole CHRIST and
Elke LORBACH

Serial No.: Unknown

Filed: February 22, 2002

For: SEQUENCE-SPECIFIC DNA RECOMBINATION IN EUKARYOTIC CELLS

Group Art Unit: Unknown

Examiner: Unknown

Atty. Dkt. No.: DEBE:008US/SLH

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Please consider the following amendments prior to examination of the above-captioned application. It is believed that no fees are occasioned by this filing; however, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason, the Commissioner is authorized to deduct said fees from Fulbright & Jaworski L.L.P. Account No.: 50-1212/10201861/SLH. Please date stamp and return the enclosed postcard as evidence of receipt.

AMENDMENT

In the Specification

Please insert the following paragraph after line 1 of page 1:

This application claims priority to PCT/DE 00/02947, filed on August 29, 2000, and German DE 199 41 186.7, filed August 30, 1999. The entire content of both these applications are incorporated by reference.

Please insert page 39 with the following text:

ABSTRACT

The present invention relates to a method of sequence specific recombination of DNA in eukaryotic cells utilizing *att* sequences from the bacteriophage *lambda*. A particular embodiment of the invention relates to a method further comprising performing the sequence specific recombination of DNA with an Int and a Xis factor. The present invention further relates to vectors containing each of these sequences and their use as medicaments.

In the Claims

Please cancel claims 1-28, without prejudice or disclaimer.

Please add the following claims:

29. (New) A method of sequence specific recombination of DNA in a eukaryotic cell, comprising:

(a) providing said eukaryotic cell, said cell comprising a first DNA segment, said first DNA segment comprising an *att*B sequence according to SEQ ID NO:1 or a derivative thereof, an *att*P sequence according to SEQ ID NO:2 or a derivative thereof, an *att*L sequence according to SEQ ID NO:3 or a derivative thereof, or an *att*R sequence according to SEQ ID NO:4 or a derivative thereof;

(b) introducing a second DNA segment into said cell, wherein if said first DNA segment comprises an *att*B sequence according to SEQ ID NO:1 or a derivative

thereof, said second DNA segment comprises an *att*P sequence according to SEQ ID NO:2 or a derivative thereof, wherein if said first DNA segment comprises an *att*P sequence according to SEQ ID NO:2 or a derivative thereof, said second DNA segment comprises an *att*B sequence according to SEQ ID NO:1 or a derivative thereof, wherein if said first DNA segment comprises an *att*L sequence according to SEQ ID NO:3 or a derivative thereof said second DNA segment comprises an *att*R sequence according to SEQ ID NO:4 or a derivative thereof, or wherein if said first DNA segment comprises an *att*R sequence according to SEQ ID NO:4 or a derivative thereof said second DNA segment comprises an *att*L sequence according to SEQ ID NO:3 or a derivative thereof; and

wherein said cell further expresses a bacteriophage *lambda* integrase Int, which induces sequence specific recombination through said *att*B and *att*P or *att*R and *att*L sequences.

30. (New) The method of claim 29, wherein said first DNA segment was introduced into the genome of said cell by recombinant methods.

31. (New) The method of claim 29, wherein said first DNA segment is naturally-occurring in the genome of said cell.

32. (New) The method of claim 29, wherein said first DNA segment comprises an *att*B sequence according to SEQ ID NO:1 or a derivative thereof, and said second DNA comprises an *att*P sequence according to SEQ ID NO:2 or a derivative thereof.

33. (New) The method of claim 29, wherein said first DNA segment comprises an *att*P sequence according to SEQ ID NO:2 or a derivative thereof, and said second DNA comprises an *att*B sequence according to SEQ ID NO:1 or a derivative thereof.

34. (New) The method of claim 29, wherein said first DNA segment comprises an *att*L sequence according to SEQ ID NO:3 or a derivative thereof, and said second DNA sequence comprises an *att*R sequence according to SEQ ID NO:4 or a derivative thereof, further comprising, in step (c), providing to said cell a Xis factor.

35. (New) The method of claim 29, wherein said first DNA segment comprises an *att*R sequence according to SEQ ID NO:4 or a derivative thereof, and said second DNA sequence comprises an *att*L sequence according to SEQ ID NO:3 or a derivative thereof, further comprising, in step (c), providing to said cell a Xis factor.

36. (New) The method of claim 29, further comprising providing to said cell a third DNA segment comprising an Int gene.((Steve, the third segment should only be the Int gene, the fourth segment should be the Xis factor gene. The Int is essential for the recombination of all four att-sites, the Xis factor gene only for the reaction between attR and attL.))

37. (New) The method of claim 36, further comprising providing to said cell a fourth DNA segment comprising Xis factor gene, respectively.

38. (New) The method of claim 36, wherein said third DNA segment further comprises a regulatory sequence effecting a spatial and/or temporal expression of the Int gene. ((Steve, please take care that all possibilities of original claim 6 are claimed))

39. (New) The method of claim 37, wherein said fourth DNA segment further comprises a regulatory sequence effecting a spatial and/or temporal expression fo the Xis factor gene.

40. (New) The method of claim 29 wherein said Int is a modified integrase.

41. (New) The method of claim 37, wherein said modified Int is Int-h or Int-h/218.

42. (New) The method according to claim 29, wherein in step (c) further comprises providing an "integration host factor" (IHF).

43. (New) The method according to claim 29, wherein said first and/or second DNA segment further comprise a sequence effecting integration of said first and/or second DNA segment into the genome of said cell by homologous recombination.

44. (New) The method of claim 29, wherein said first and/or second DNA segment further comprises a sequence coding for a polypeptide of interest.

45. (New) The method of claim 44, wherein said polypeptide of interest is a structural protein, an endogenous or exogenous enzyme, a regulatory protein or a marker protein.

46. (New) The method of claim 29, wherein said first and second DNA segment are introduced into the eukaryotic cell on the same DNA molecule.

47. (New) The method of claim 29, wherein said eukaryotic cell is a mammalian cell.

48. (New) The method of claim 47, wherein said mammalian cell is a human, simian, mouse, rat, rabbit, hamster, goat, bovine, sheep or pig cell.

49. (New) The method of claim 29, further comprising:

(d) performing a second sequence specific recombination of DNA by an Int and a Xis factor after the steps (a)-(c), wherein said first DNA sequence comprises said *att*B sequence according to SEQ ID NO:1 or a derivative thereof and said second DNA sequence comprises the *att*P sequence according to SEQ ID NO:2 or a derivative thereof, or wherein said first DNA sequence comprises said *att*P sequence according to SEQ ID NO:2 or a derivative thereof and said second DNA sequence comprises the *att*B sequence according to SEQ ID NO:1 or a derivative thereof.

50. (New) The method of claim 49, further introducing a further DNA sequence into said cells, the further DNA sequence comprising a Xis factor gene.

51. (New) The method of claim 50, wherein said further DNA sequence comprises further a regulatory DNA sequence effecting a spatial and/or temporal expression of said Xis factor gene.

52. (New) The method of claim 29, wherein said method is performed in a vertebrate organism.

53. (New) The method of claim 52, wherein said vertebrate organism is a human.

54. (New) A nucleic acid comprising the sequence of SEQ ID NO:5, or a derivative thereof having as many as six substitutions, with the provision that the derivative is not the wild-type *att*P sequence.

55. (New) A vector comprising:

(a) a nucleic acid segment comprising the sequence of SEQ ID NO:5, or a derivative thereof having as many as six substitutions, with the provision that the derivative is not the wild-type *att*P sequence; and

(b) a nucleic acid segment coding for a selected gene or a fragment thereof.

56. (New) The vector of claim 53, wherein said selected gene is the CFTR gene, ADA gene, LDL receptor gene, ß globin gene, Factor VIII gene or Factor IX gene, alpha-1-antitrypsin gene or the dystropin gene or a gene fragment of one of said genes.

57. (New) The vector of 53, further comprising a nucleic acid segment comprising a regulatory element.

58. (New) A eukaryotic cell obtainable according to the method of claim 29.

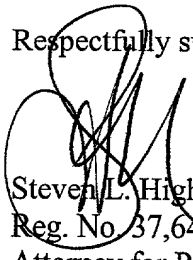
59. (New) A non-human transgenic organism comprising at least one cell made according to the method of claim 29.

60. (New) The organism according to claim 54, wherein said organism is a mouse, rat rabbit or hamster.

REMARKS

Should the examiner have any questions regarding the content of this preliminary amendment, a telephone call to the undersigned is invited.

Respectfully submitted,



Steven L. Highlander
Reg. No. 37,642
Attorney for Peter Droge,
Nicole Christ and Elke Lorbach

FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 536-3184

Date: February 25, 2002